REPORT

ASSESSMENT OF ACUTE DERMAL TOXICITY WITH

[redacted]

IN THE RAT

NOTOX Project 338671
NOTOX Substance 111834/B

CONFIDENTIALITY STATEMENT

This report contains the unpublished results of research sponsored by [redacted] B.V. Reproduction, issue or disclosure to third parties in any form is not permitted without prior written authorization from the sponsor.

STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice which are essentially in conformity with:

United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

Japanese Ministry of Agriculture, Forestry and Fisheries. 59 NohSan, Notifications No. 3850.

Japanese Ministry of Economy, Trade and Industry. Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85.

Japanese Ministry of Health, Labor and Welfare. Ordinance No.21.

Study Director:
[redacted]

.....

Date: 04 February 2002.

Management:
[redacted]M

.....

Date: 4 February 2002

QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was audited by the NOTOX Quality Assurance Unit to ensure that the methods and results accurately reflect the raw data.

The dates of Quality Assurance inspections and audits are given below.
During the on-site inspections procedures applicable to this type of study were inspected.

DATES OF QAU INSPECTIONS/ AUDITS	REPORTING DATES
on-site inspection(s)	
08-16 October 2001 (Process)	25 October 2001
27-30 November 2001 (Process)	03 December 2001
protocol inspection(s)	
08 November 2001 (Study)	08 November 2001
report audit(s)	
14 January 2002 (Study)	14 January 2002

Head of Quality Assurance: [redacted].

Date: 5-2-02

SUMMARY

Assessment of acute dermal toxicity with [redacted] in the rat.

The study was carried out based on the guidelines described in: EC Commission Directive 92/69/EEC, Part B.3, "Acute Toxicity-Dermal" and OECD No.402, "Acute Dermal Toxicity".

TRIGONOX R-938 was administered to five rats of each sex by dermal application at 2000 mg/kg body weight for 24 hours. Animals were subjected to daily observations and weekly determination of body weight. Macroscopic examination was performed after terminal sacrifice (day 15).

No mortality occurred.

Chromodacryorrhoea was noted among the animals between days 2 and 6. One male and one female showed hunched posture on day 2.

Maculate or general erythema, scales, fissures, scabs, necrosis and/or wounds were seen in the treated skin-area of the animals during the observation period. One male showed brown staining of the head on day 1.

The changes noted in body weight gain in males and females were within the range expected for rats used in this type of study and were therefore considered not indicative of toxicity.

Most males showed isolated scab formation on the treated skin. No further abnomalities were found at macroscopic post mortem examination of the animals.

The dermal LD_{50} value of [redacted] in Wistar rats was established to exceed 2000 mg/kg body weight.

Based on these results and according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC), [redacted] or dermal toxicity.

PREFACE

Sponsor	[redacted]
Study Monitor	[redacted] SHERA, Regulatory Affairs
Testing Facility	NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch The Netherlands
Study Director	[redacted]
Study Plan	Start : 22 November 2001 End : 06 December 2001

TEST SUBSTANCE

The sponsor is responsible for all test substance data unless determined by NOTOX.

Identification	[redacted]
Chemical name	[redacted]
CAS RN	[redacted]
[redacted]	Clear colourless liquid
Batch	1510-14
Purity	See Certificate of Analysis
Test substance storage	In refrigerator in the dark
Stability under storage conditions	Stable
Expiry date	01 January 2003
Density	Approx. 1160 $kg.m^{-3}$

TEST SUBSTANCE PREPARATION

The test substance was dosed undiluted as delivered by the sponsor.

PURPOSE AND RATIONALE

The objective of this study was to assess the toxicity of the test substance when administered to rats as a single dermal application.
This study should provide a rational basis for risk assessment in man.
The dermal route was selected as it is a possible route of human exposure during manufacture, handling or use of the test substance.

GUIDELINES

As required by the Dutch Act on Animal Experimentation, the study protocol was reviewed and agreed by the Article 14-functionary and the Ethical Committee of NOTOX (DEC NOTOX 97-03-13) as required by the Dutch Act on Animal Experimentation (February 1997). The study procedures described in this report were based on the following guidelines:

European Community (EC), Council Directive 67/548/EEC, as last amended by Commission Directive 92/69/EEC, Annex V, Part B, Methods for the determination of Toxicity, B.3: "Acute Toxicity-Dermal". Official Journal of the European Communities No. L 383, 1992.

Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Section 4, Health Effects, No. 402, "Acute Dermal Toxicity", Paris Cedex, 1987.

ARCHIVING

NOTOX B.V. will archive for at least 10 years raw data, protocol, report and test substance reference sample. No data will be withdrawn without the sponsor's written consent.

TEST SYSTEM

Species	Rat, Wistar strain Crl:(WI) BR (outbred, SPF-Quality). Recognised by international guidelines as the recommended test system (e.g. OECD, EC). Source : Charles River Deutschland, Germany.
Number of animals	5 males and 5 females (females were nulliparous and non-pregnant).
Age and body weight	Young adult animals (approx. 11 weeks old) were selected. Body weight variation did not exceed +/- 20% of the sex mean.
Identification	Earmark

ANIMAL HUSBANDRY

Conditions
A controlled environment was maintained in the room with optimal conditions considered as being approximately 15 air changes per hour, a temperature of 21±3°C, a relative humidity of 30-70% and 12 hours artificial fluorescent light and 12 hours dark per day.
Deviations from the maximum level for relative humidity (with a maximum of 7%) occurred which might have been caused by cleaning procedures in the room. Based on laboratory historical data these deviations were considered not to have affected the study integrity.

Accommodation
Individually housed in labelled polycarbonate cages (type III, height 15 cm.) containing purified sawdust as bedding material (SAWI, Jelu Werk, Rosenberg, Germany). Certificates of analysis were examined and then retained in the NOTOX archives.
Acclimatisation period was at least 5 days before start of treatment under laboratory conditions.

Diet
Free access to standard pelleted laboratory animal diet (from Altromin (code VRF 1), Lage, Germany). Certificates of analysis were examined and then retained in the NOTOX archives.

Water
Free access to tap-water. Certificates of quarterly analysis were examined and then retained in the NOTOX archives.

TREATMENT

A health inspection was performed prior to commencement of treatment, to ensure that the animals were in a good state of health. Special attention was paid to the skin to be treated, which was intact and free from any abnormality.

Method	Dermal application.
Clipping	One day before exposure (day -1) an area of approximately 5x7 cm on the back of the animal was clipped.
Application	The test substance was applied in an area of approx. 10% of the total body surface, i.e. approx. 25 cm^2 for males and 18 cm^2 for females. The test substance was held in contact with the skin with a dressing, consisting of a surgical gauze (Surgy 1D)*, successively covered with aluminium foil and Coban flexible bandage*. A piece of Micropore tape* was additionally used for fixation of the bandages in females only. *. Manufacturers: Laboratoires Stella s.a., Liege, Belgium (surgical gauze) and 3M, St. Paul, Minnesota, U.S.A. (Coban & Micropore).
Frequency	Single dosage, on day 1.
Dose level (volume)	2000 mg/kg (1.72 ml/kg) body weight. Dose volume calculated as follows: dose level : density.
Application period	24 hours, after which dressings were removed and residual test substance removed using a tissue moistened with water.

OBSERVATIONS

Mortality/Viability	Twice daily.
Body weights	Days 1 (pre-administration), 8 and 15.
Clinical signs	At periodic intervals on the day of dosing (day 1) and once daily thereafter, until day 15. The time of onset, degree and duration were recorded and the symptoms graded according to fixed scales: Maximum grade 4: grading slight (1) to very severe (4) Maximum grade 3: grading slight (1) to severe (3) Maximum grade 1: presence is scored (1).
Necropsy	All animals surviving to the end of the observation period (day 15) were sacrificed by asphyxiation using an oxygen/carbon dioxide procedure. All animals assigned to the study were subjected to necropsy and descriptions of all internal macroscopic abnormalities recorded.

INTERPRETATION

No statistical analysis was performed.

The results were evaluated according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC).

RESULTS

Dose level	Sex	Date of treatment
2000 mg/kg	females	22 November 2001
2000 mg/kg	males	22 November 2001

Mortality
No mortality occurred.

Clinical Signs (Table 1)
Chromodacryorrhoea was noted among the animals between days 2 and 6. One male and one female showed hunched posture on day 2.

Maculate or general erythema, scales, fissures, scabs, necrosis and/or wounds were seen in the treated skin-area of the animals during the observation period. One male showed brown staining of the head on day 1.

Body Weight (Table 2)
The changes noted in body weight gain in males and females were within the range expected for rats used in this type of study and were therefore considered not indicative of toxicity.

Macroscopic Findings (Table 3)
Most males showed isolated scab formation on the treated skin. No further abnomalities were found at macroscopic post mortem examination of the animals.

CONCLUSION

The dermal LD_{50} value of [redacted] in Wistar rats was established to exceed 2000 mg/kg body weight.

Based on these results and according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC), [redacted] for dermal toxicity.

TABLE 1 : CLINICAL SIGNS

TEST DAY		1	1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
HOURS AFTER TREATMENT	MAX GRADE	0	2	4														
MALES 2000 MG/KG																		
ANIMAL 1																		
SKIN / FUR / PLUMAGE																		
ERYTHEMA MACULATE (TREATED SKIN)	(4)	-	-	-	3	3	3	3	3	2	2	2	2	2	2	2	2	2
NECROSIS (TREATED SKIN)	(3)	-	-	-	2	2	3	2	2	-	-	-	-	-	-	-	-	-
FISSURES (TREATED SKIN)	(3)	-	-	-	-	-	-	2	2	2	1	1	1	1	-	-	-	-
SCALES (TREATED SKIN)	(3)	-	-	-	-	-	-	2	2	2	2	2	2	2	2	2	2	2
SCABS (TREATED SKIN)	(3)	-	-	-	-	2	3	2	2	2	2	2	2	2	2	2	2	2
WOUND (TREATED SKIN)	(3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
ANIMAL 2																		
SKIN / FUR / PLUMAGE																		
ERYTHEMA MACULATE (TREATED SKIN)	(4)	-	-	-	1	1	1	1	1	1	-	-	-	-	-	-	-	-
NECROSIS (TREATED SKIN)	(3)	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-
SCALES (TREATED SKIN)	(3)	-	-	-	-	-	-	3	2	1	1	1	-	-	-	-	-	-
BROWN STAINING (HEAD)	(1)	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SECRETION / EXCRETION																		
CHROMODACRYORRHOEA (HEAD)	(3)	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
ANIMAL 3																		
POSTURE																		
HUNCHED POSTURE	(1)	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
SKIN / FUR / PLUMAGE																		
GENERAL ERYTHEMA (TREATED SKIN)	(4)	-	-	-	3	3	2	2	1	1	1	1	1	1	1	1	1	1
NECROSIS (TREATED SKIN)	(3)	-	-	-	2	2	1	1	-	-	-	-	-	-	-	-	-	-
FISSURES (TREATED SKIN)	(3)	-	-	-	-	-	-	2	2	1	-	-	-	-	-	-	-	-
SCALES (TREATED SKIN)	(3)	-	-	-	-	-	-	2	2	2	2	1	1	1	1	1	1	1
SCABS (TREATED SKIN)	(3)	-	-	-	-	-	-	-	-	-	1	1	1	1	1	1	-	-
SECRETION / EXCRETION																		
CHROMODACRYORRHOEA (NOSE)	(3)	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-
ANIMAL 4																		
SKIN / FUR / PLUMAGE																		
GENERAL ERYTHEMA (TREATED SKIN)	(4)	-	-	-	3	2	2	2	2	1	1	1	1	1	1	1	1	1
NECROSIS (TREATED SKIN)	(3)	-	-	-	1	1	1	1	-	-	-	-	-	-	-	-	-	-
FISSURES (TREATED SKIN)	(3)	-	-	-	-	-	-	2	2	2	1	1	1	1	-	-	-	-
SCALES (TREATED SKIN)	(3)	-	-	-	-	-	-	2	2	2	2	2	2	2	1	1	1	1
SCABS (TREATED SKIN)	(3)	-	-	-	-	-	1	1	1	1	2	2	2	2	2	2	2	2
ANIMAL 5																		
SKIN / FUR / PLUMAGE																		
ERYTHEMA MACULATE (TREATED SKIN)	(4)	-	-	-	2	1	1	2	2	2	1	1	1	1	1	1	1	1
NECROSIS (TREATED SKIN)	(3)	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-
FISSURES (TREATED SKIN)	(3)	-	-	-	-	-	-	2	2	2	-	-	-	-	-	-	-	-
SCALES (TREATED SKIN)	(3)	-	-	-	-	-	-	2	2	2	2	2	2	2	1	1	1	1
SCABS (TREATED SKIN)	(3)	-	-	-	-	-	-	1	1	1	1	1	1	1	1	1	1	1
SECRETION / EXCRETION																		
CHROMODACRYORRHOEA (NOSE)	(3)	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
FEMALES 2000 MG/KG																		
ANIMAL 6																		
SKIN / FUR / PLUMAGE																		
GENERAL ERYTHEMA (TREATED SKIN)	(4)	-	-	-	-	-	-	2	2	1	1	1	1	1	-	-	-	-
ERYTHEMA MACULATE (TREATED SKIN)	(4)	-	-	-	2	1	1	-	-	-	-	-	-	-	-	-	-	-
SCALES (TREATED SKIN)	(3)	-	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-
SECRETION / EXCRETION																		
CHROMODACRYORRHOEA (HEAD)	(3)	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-
CHROMODACRYORRHOEA (NECK)	(3)	-	-	-	-	-	-	2	1	-	-	-	-	-	-	-	-	-

- = SIGN NOT OBSERVED / . = OBSERVATION NOT PERFORMED / + = ANIMAL DEAD

TABLE 1 : CLINICAL SIGNS

TEST DAY		1	1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
HOURS AFTER TREATMENT	MAX GRADE	0	2	4														
FEMALES 2000 MG/KG																		
ANIMAL 7																		
POSTURE																		
HUNCHED POSTURE	(1)	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
SKIN / FUR / PLUMAGE																		
GENERAL ERYTHEMA (TREATED SKIN)	(4)	-	-	-	1	2	2	2	2	1	1	1	1	1	1	1	-	-
SCALES (TREATED SKIN)	(3)	-	-	-	-	-	-	2	2	2	2	2	1	1	-	-	-	-
SCABS (TREATED SKIN)	(3)	-	-	-	-	1	1	1	1	1	1	1	-	-	-	-	-	-
ANIMAL 8																		
SKIN / FUR / PLUMAGE																		
GENERAL ERYTHEMA (TREATED SKIN)	(4)	-	-	-	2	2	1	2	2	1	1	1	1	1	1	1	1	1
NECROSIS (TREATED SKIN)	(3)	-	-	-	2	2	1	1	-	-	-	-	-	-	-	-	-	-
SCALES (TREATED SKIN)	(3)	-	-	-	-	-	-	2	2	2	2	1	1	1	1	1	1	1
SCABS (TREATED SKIN)	(3)	-	-	-	-	-	-	-	1	1	2	2	2	2	2	2	1	-
ANIMAL 9																		
SKIN / FUR / PLUMAGE																		
GENERAL ERYTHEMA (TREATED SKIN)	(4)	-	-	-	3	3	3	2	2	2	2	2	1	1	1	1	-	-
FISSURES (TREATED SKIN)	(3)	-	-	-	-	-	-	2	2	2	-	-	-	-	-	-	-	-
SCALES (TREATED SKIN)	(3)	-	-	-	-	-	-	1	1	1	2	1	1	1	1	1	-	-
SCABS (TREATED SKIN)	(3)	-	-	-	-	-	1	2	2	1	-	-	-	-	-	-	-	-
SECRETION / EXCRETION																		
CHROMODACRYORRHOEA (NOSE)	(3)	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-
ANIMAL 10																		
SKIN / FUR / PLUMAGE																		
GENERAL ERYTHEMA (TREATED SKIN)	(4)	-	-	-	1	1	1	1	1	1	-	-	-	-	-	-	-	-

TABLE 2 : BODY WEIGHTS (GRAM)

SEX/DOSE LEVEL	ANIMAL	DAY 1	DAY 8	DAY 15
MALES 2000 MG/KG				
	1	436	427	453
	2	420	430	459
	3	484	490	541
	4	351	351	371
	5	384	387	412
	MEAN	415	417	447
	ST.DEV.	51	52	63
	N	5	5	5

- = SIGN NOT OBSERVED / . = OBSERVATION NOT PERFORMED / + = ANIMAL DEAD

TABLE 2 : BODY WEIGHTS (GRAM)

SEX/DOSE LEVEL	ANIMAL	DAY 1	DAY 8	DAY 15
FEMALES 2000 MG/KG				
	6	274	283	291
	7	257	246	263
	8	281	270	285
	9	278	289	297
	10	268	274	273
	MEAN	272	272	282
	ST.DEV.	10	17	14
	N	5	5	5

TABLE 3 : MACROSCOPIC FINDINGS

ANIMAL	ORGAN	FINDING	DAY OF DEATH
MALES 2000 MG/KG			
1		No findings noted	Scheduled necropsy Day 15 after treatment
2	Treated skin	Scab formation, isolated.	Scheduled necropsy Day 15 after treatment
3	Treated skin	Scab formation, isolated.	Scheduled necropsy Day 15 after treatment
4	Treated skin	Scab formation, isolated.	Scheduled necropsy Day 15 after treatment
5	Treated skin	Scab formation, isolated.	Scheduled necropsy Day 15 after treatment
FEMALES 2000 MG/KG			
6		No findings noted	Scheduled necropsy Day 15 after treatment
7		No findings noted	Scheduled necropsy Day 15 after treatment
8		No findings noted	Scheduled necropsy Day 15 after treatment
9		No findings noted	Scheduled necropsy Day 15 after treatment
10		No findings noted	Scheduled necropsy Day 15 after treatment

[redacted]

[redacted]

Certificate of Analysis

Polymer Chemicals

TNA-2001007
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ICS-331

Product name	: [redacted]
Chemical name	: [redacted]
Batch number	: **1510-14**

Test results:

Method	Analysis of	Unit	Result *1
Jo/72.11, [redacted]	[redacted] *See page 2 for a specification*		
J20010792	D[redacted]e		
J20010792	[redacted]	% m/m	2.0 (± 0.3)
Amp/88.9	Water	% m/m	2.6 (± 0.3)
J20010792	Unidentified impurities	% m/m	0.5 (± 0.2)

*1 bracketed values are estimated 95% confidence intervals

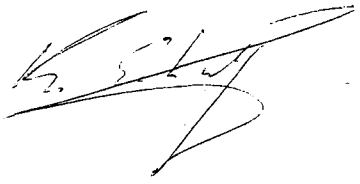
File code : **TNA-2001007**

Analytical documentation : 20010792

Authorized by

Name : [redacted]
Function : Section Head, Analytical Research Department
Date : October 25, 2001

Signature :



[redacted]

[redacted]

Certificate of Analysis [redacted]

[redacted]s

structure	% m/m
 (Type IV) [redacted]	18.6
 (Type III) [redacted]	7.9
[redacted]	2.1

[redacted]